Volume 16: 165–174
Publication date: 11 November 2014
dx.doi.org/10.7751/telopea20147809

TELOPEA
Journal of Plant Systematics

The Royal Botanic Gardens & Domain Trust

plantnet.rbgsyd.nsw.gov.au/Telopea • escholarship.usyd.edu.au/journals/index.php/TEL • ISSN 0312-9764 (Print) • ISSN 2200-4025 (Online)

Thismia megalongensis (Thismiaceae), a new species of *Thismia* from New South Wales

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Abstract

The new species *Thismia megalongensis* (Thismiaceae) from New South Wales, Australia, is described, illustrated and compared with other Australian species. A key to Australian species of *Thismia* is included. Habitat details, conservation status, and phylogenetic affinities of the new species are discussed.

Introduction

Thismia Griffith is a genus comprising c. 50 species of small, achlorophyllous, myco-heterotrophic herbs mostly from tropical rainforest in America and Asia.

Three species are currently described from temperate areas in the southern hemisphere, *T. rodwayi* F.Muell. and *T. clavarioides* K.Thiele & P.Jordan from south-eastern Australia and *T. hillii* (Cheeseman) N.Pfeiff. in New Zealand. Until recently the latter species was classified as *T. rodwayi*, which it resembles closely based on the description (Cheeseman 1908; Schlechter 1921). Its recognition as a separate species was proposed to retain a monophyletic *T. rodwayi* (Merckx and Smets 2014). There is also a third species known in Australia, *Thismia yorkensis* Cribb, currently known from Cape York Peninsula in northern Queensland.

Thismia is included in Burmanniaceae by several authors including the Angiosperm Phylogeny Group (APG 2009). However, molecular phylogenetic evidence indicates that *Thismia* is not part of Burmanniaceae, but should be placed with the mycoheterotrophic genera *Tiputinia* P.E.Berry & C.L.Woodw., *Haplothismia* Airy Shaw, and *Oxygyne* Schltr. as the sister group of *Tacca* J.R.Forst. & G.Forst. (Merckx et al. 2006, 2009; Merckx & Smets 2014). Following these results *Thismia*, *Tiputinia*, *Haplothismia*, and *Oxygyne* form the family Thismiaceae *sensu stricto* (without *Afrothismia* Schltr.). The family Thismiaceae is also recognised by the National Herbarium of New South Wales (NSW).

In January 2011 one of us (CH) collected a flowering specimen of *Thismia* from warm temperate rainforest near Blackheath in the Blue Mountains of New South Wales. Many more flowering specimens (c. 400) were subsequently found during a search of the surrounding area. Dissections of the flowering material and comparison with specimens of *T. rodwayi* and *T. clavarioides* show that the Blackheath plants comprise a new species, here described as *Thismia megalongensis*.

Materials and methods

Plant materials—Multiple specimens of the new species were collected in spirit, CTAB, and silica gel at the Megalong Valley. For comparison, specimens of *T. rodwayi* were collected from the Grose Valley at Blackheath, and specimens of *T. rodwayi* and *T. clavarioides* from Morton National Park (the type locality of the latter species) near Bundanoon. Voucher specimens are deposited at NSW.

Analyses of molecular data—Appendix 1 lists all taxa included in the molecular phylogenetic analysis with GenBank accession numbers and voucher information for newly sequenced taxa. For sequences obtained for this study, DNA was extracted from silica-dried or CTAB preserved specimens with the DNeasy Plant Mini Kit (QIAGEN, Venlo, the Netherlands) following the manufacturer's instructions. Amplification of the 18S rDNA region was carried out as described in Merckx et al. (2006). Mitochondrial *atp1* sequences were amplified with the primers and conditions described in Eyre-Walker and Gaut (1997). Sanger sequencing was performed by the Macrogen sequencing facilities (Amsterdam, the Netherlands). Sequences were assembled and edited with Geneious Pro v5.5.6 (Biomatters, New Zealand). Sequences were aligned using the MAFFT v.6.814b alignment tool (Katoh et al. 2002) implemented in Geneious Pro. The substitution model for both DNA regions was selected with jModeltest v2.3.1 (Darriba et al. 2012) under the Akaike Information Criterion (AIC). The best-fitting model for the 18S rDNA and *atp1* datasets are respectively the GTR+I+G model and the GTR+G model. Bayesian inference analyses were conducted using MrBayes v.3.2.1 (Ronquist et al. 2012). Each of the DNA datasets was analyzed separately, and also combined using a separate model for each of the two data partitions as determined by jModeltest. Analysis comprised two parallel analyses each consisting of four Markov chains starting with a random tree and running simultaneously for 5×10^6 generations. Trees were sampled every 5000th generation. The first 250 sampled trees of each analysis were regarded as 'burn in' and discarded. A majority rule consensus tree was calculated using the remaining 1500 trees.

Habitat assessment – Habitats at the collecting sites were assessed using a 20m × 20m square quadrat, recording all vascular plant species in terms of vertical coverage of spatial area ('cover') and frequency in bands of numbers representing minimum counts of observed individuals ('abundance'), as well as a range of biotic and abiotic environmental variables, in compliance with the NSW Interim Type Standard (Sivertsen 2009). Data was captured in the Vegetation Information System VIS Flora Survey module of the NSW Bionet nature records system (www.bionet.nsw.gov.au) for archiving. Vegetation communities in which the *Thismia* samples were found are compared to the state-wide list of NSW Plant Community Types maintained in the VIS , these having been defined using the standardised quadrat and assessment method, with data captured using the standardised method to allow analytical comparison of the sites with the nominated communities.

Results

Morphological observations – *Thismia megalongensis* (Figs 1, 3a,b) differs from *T. clavarioides* (Fig. 3c,d) in having a richly coloured perianth, shorter, thinner apical awns on the inner perianth segments, thinner bristles on the outer perianth, and a fine rounded end to the anther bristle. In contrast *T. clavarioides* is characterized by a mostly colourless perianth, long, thick apical awns on the inner perianth segments, thicker outer perianth lobe bristles, and an anther bristle terminating in a club-shaped group of papillae.

It differs from *T. rodwayi* (Fig. 3e,f) and *T. hilli* by having finer, more elongated apical awns (Figs 1, 3a,b), and by the presence lateral bristle developed from each outer perianth segment (Figs 1, 3a,b, 4a). In *T. rodwayi* and *T. hillii* bristles on the outer perianth segments are lacking. And in *T. rodwayi* apical awns are very short (Figs 3e,f, 4b).

Phylogenetic analyses – No significantly supported incongruences were observed between the Bayesian 18S rDNA and *atp1* topologies. Bayesian analysis on the combined data resulted in a very similar, but better supported topology, shown in Fig. 5. As found in previous analyses (e.g. Merckx et al. 2009; Merckx and Smets 2014) *Thismia* is not a monophyletic group due to the inclusion of *Haplothismia* and *Tiputinia.* All included Old World *Thismia* species (*Thismia* subgen. *Thismia*) are part of a well-supported monophyletic group (Bayesian Posterior Probability = 99.9). Within this clade the two species from Taiwan, *T. taiwanensis* Sheng Z.Yang, R.M.K.Saunders & C.J.Hsu and *T. huangii* P.Y.Jiang & T.H.Hsieh are well-supported sister taxa. This clade is sister to a clade that consists of taxa from Borneo (*T. clavigera* F.Muell. and *T. aseroe* Becc.), and Australia and New Zealand. Within the latter clade *T. clavarioides* is the sister species of *T. rodwayi,* while *T. megalongensis* is the sister species of *T. hillii* from New Zealand. (Fig. 5).

Thus, the phylogenetic analysis based on molecular data suggests that *T. megalongensis* is most closely related to *T. hillii* from New Zealand. A population genetic study is currently being carried out by the authors to explore the population structure and genetic differences between the populations and species of *Thismia* in Australia and New Zealand.

Taxonomic treatment

Thismia megalongensis C.Hunt, G.Steenbeeke & V.Merckx ***sp. nov.***

Similis *T. rodwayi*, sed differt per tenues exteriores perianthium setis et extense awns ad apicem. Similar to *T. rodwayi*, but differs by the thin outer perianth bristles and extended awns at the apex.

Holotype: Australia, New South Wales, Central Tablelands: Mermaids Cave Recreation Reserve, near Coachwood Glen walk, Blackheath, 17 Jan 2011, C. *Hunt & G. Steenbeeke s.n.* (NSW889649 – 70% ethyl alcohol).

Achlorophyllous mycoheterotrophic herb of the litter layer and the upper mineral soil, with creeping vermiform 1–2 mm thick rhizome that infrequently branches. Leaves absent; colourless bracts present on the peduncle, to 7 mm long, the uppermost clasping the base of the flower. Flowers solitary, 15–25 mm long (including the dorsal awns on the inner perianth lobes), usually fully emergent above the soil, with the base of the flower at the same level as the litter or occasionally below the uppermost layer, solitary on a short to elongate scape which is 1–2 mm wide and 10–60 mm in length. Perianth urceolate, 10–16 mm long, 4–6 mm wide, white at the base, rich orange from near base to apex, comprising 6 segments fused for the basal ¾ of their length, the upper part of the segments forming an open 3-way arch or mitre in the apical ¼. Perianth segments with a prominent central rib, each segment fused along the margin to the adjacent segment with an elongate region of thickened darker orange tissue, inner and outer surfaces irregularly rugose. Outer perianth segments each forming an abaxial lobe out from the perianth tube, with a prominent bristle developing from the central rib and extending outwards beyond the apex of the lobe, (2–)3–6(–8) mm long. Inner perianth segments connate at the top forming a mitre, with an apical awn developing from the central rib on the outer surface of each. Awns extend beyond the mitreform apex, decumbent to near erect, often curved, (3–)4–6(–9) mm long. Stamens hanging as a prominent 6-lobed annulus, deep orange, with a central rib, ending in a distal, two lobed, skirt and inner petticoat-like appendage with a fine straight or curved bristle. Anthers extrorse, with white pollen in two shallow elliptical loculi. Style short, with 3 elliptical lobes at apex. Ovary inferior, truncate to domed, 1-locular; placentas 3, free, joined at apex and base of the locule. Fruit ovoid, with a raised rim around the apical edge, c. 4 mm in diameter, fleshy, whitish. Seeds globoid to ellipsoid, golden-brown, 0.25–0.3 mm. Figs 1–4.

Derivation of epithet: derived from the type location, Megalong Valley, west of Sydney, in the upper Blue Mountains of New South Wales.

Specimens examined: *Hunt and Steenbeeke s.n.* (NSW889649 – type; NSW889871). These represent several collections made by the authors from the type location.

Fig. 1. *Thismia megalongensis* against 1mm increment rule

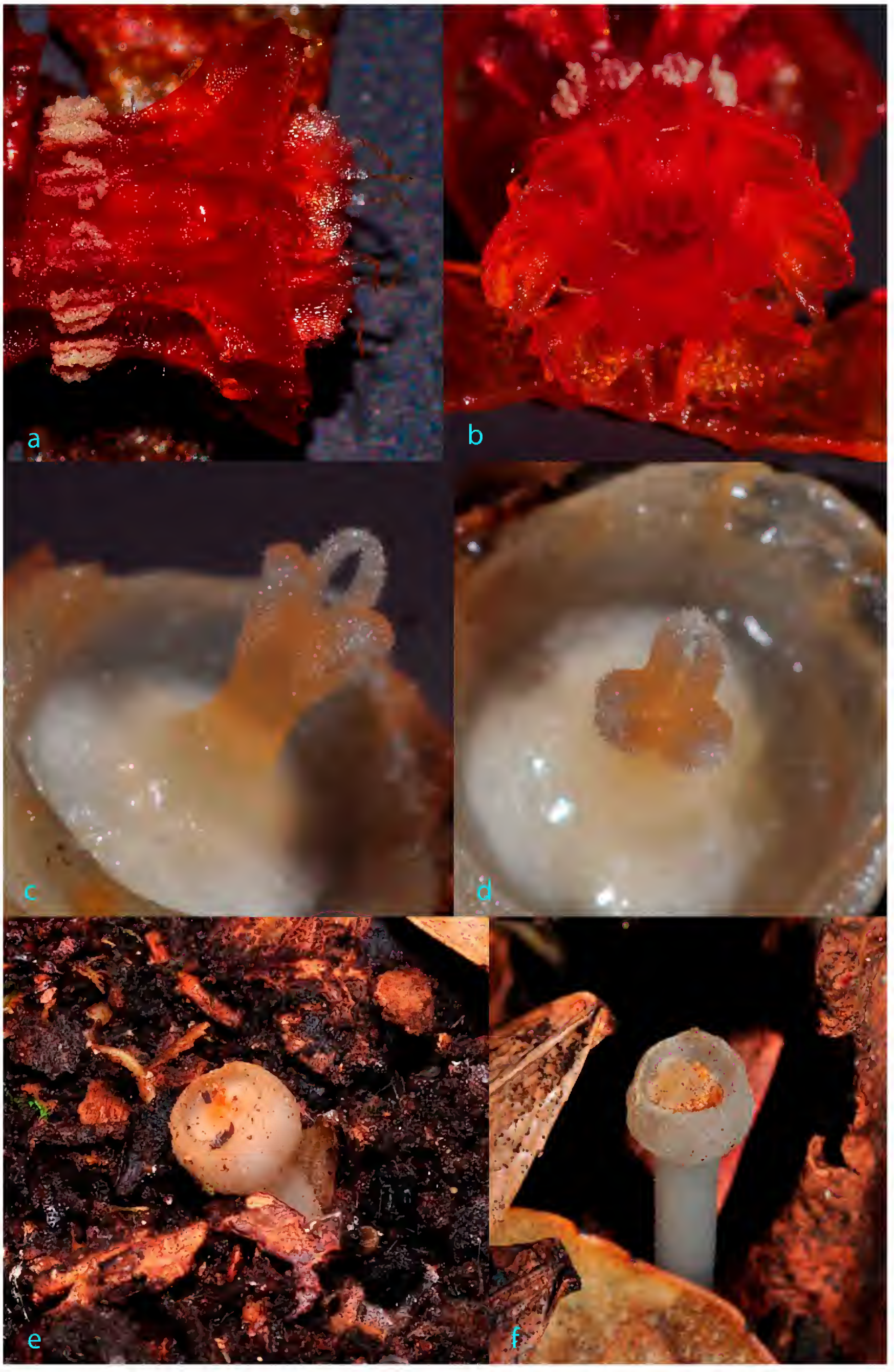

Fig 2. *Thismia megalongensis,* **a,** Anther assembly. **b,** Anther skirt assembly. **c,** Style from side. **d,** Style from above. **e,** Immature fruit. **f,** Fruit at maturity, with seeds visible.

Fig. 3. a, b, *Thismia megalongensis, in situ,* Mermaids Cave Recreation Reserve, Blackheath, as generally occurring, protruding from litter. **c,***Thismia clavarioides, in situ,* Fairy Bower Falls. **d,** *Thismia clavarioides, in situ,* East of Fairy Bower Falls, Bundanoon, both with litter removed. **e,** *Thismia rodwayi, in situ,* Fairy Bower Falls, Bundanoon, **f,** *Thismia rodwayi, in situ,* Grose Valley, Blackheath, both with litter removed.

Fig. 4. a, *Thismia megalongensis,* Lobe of outer perianth showing bristle. **b,** *Thismia rodwayi,* Lobe of outer perianth.

Distribution and habitat

Thismia megalongensis occurs in closed forest dominated by mesophyll species (Figure 6). The vegetation class is Warm Temperate Rainforest (Keith 2004) and the community is a Tall Closed Forest, NSW Plant Community Type 769 or 1529 or 1896 (OEH 2014). Dominant canopy species include *Doryphora sassafras* Endl. and *Ceratopetalum apetalum* D.Don, with emergent *Eucalyptus deanei* Maiden. A shorter tree canopy is dominated by *Acacia elata* Benth. and *Dicksonia Antarctica* Labill. The midstorey is largely absent, although saplings of the main canopy species are scattered throughout. Epiphytes are common in the upper canopy levels, dominated by orchids such as *Sarcochilus falcatus* R.Br. and *Dendrobium fairfaxii* F.Muell. & Fitzg. Stems of *Parsonsia* and *Cissus* form thin to thick lianas that often stretch from the ground well into the canopy. The groundcover is moderately dense but patchy, dominated by *Blechnum wattsii* Tindale with very occasional *Sticherus lobatus* N.A.Wakef. and moist-environment forbs (mainly *Plantago* and *Hydrocotyle*). Frequent rocky outcrops (derived from blocks from the cliff lines above) are largely covered by an array of cryptogams (mainly mosses) and creeping ferns (*Microsorum* and Hymenophyllaceae). Litter is continuous, dominated by fallen foliage and occasional larger branches and fallen logs. The depth of the litter layer is variable, but generally about 3 cm deep. Soil underneath the litter is an organic-rich, humic earth, with moderate sand component as a result of the washing in and blowing in of sands from erosion above the site.

The site in which the two other *Thismia* species occur at Bundanoon is also a closed-canopy forest with sandstone cliffs above, but is not as strongly dominated by the *Doryphora sassafras* and *Ceratopetalum apetalum*. While the former is still a prominent component of the canopy in the areas immediately surrounding the *Thismia* sites, the canopy immediately over the site investigated was dominated by *Eucryphia moorei* F.Muell. and *Tristaniopsis laurina* (Sm.) Peter G.Wilson & J.T.Waterh. making the community more indicative of Plant Community Type 767 or 1292 (OEH 2014). Canopy density was similar, and the understorey likewise dominated by ferns and with abundant litter.

Ecology

Plants were observed in the field over three consecutive summers. These observations show that *T. rodwayi* and *T. clavarioides* begin flowering earlier than *T. megalongensis*. Flowers of *T. megalongensis* have a fungal odour when fresh, and once they start to degrade the odour is similar to that of 'over-mature fish'. No insects were observed visiting the flowers. *Thismia rodwayi* has been shown to grow on specific arbuscular mycorrhizal fungi (Glomeromycota) that are likely to be simultaneously mycorrhizal with roots of surrounding trees (Merckx et al. 2012). Molecular identification of the fungal symbionts of *T. megalongensis* and related species is currently ongoing.

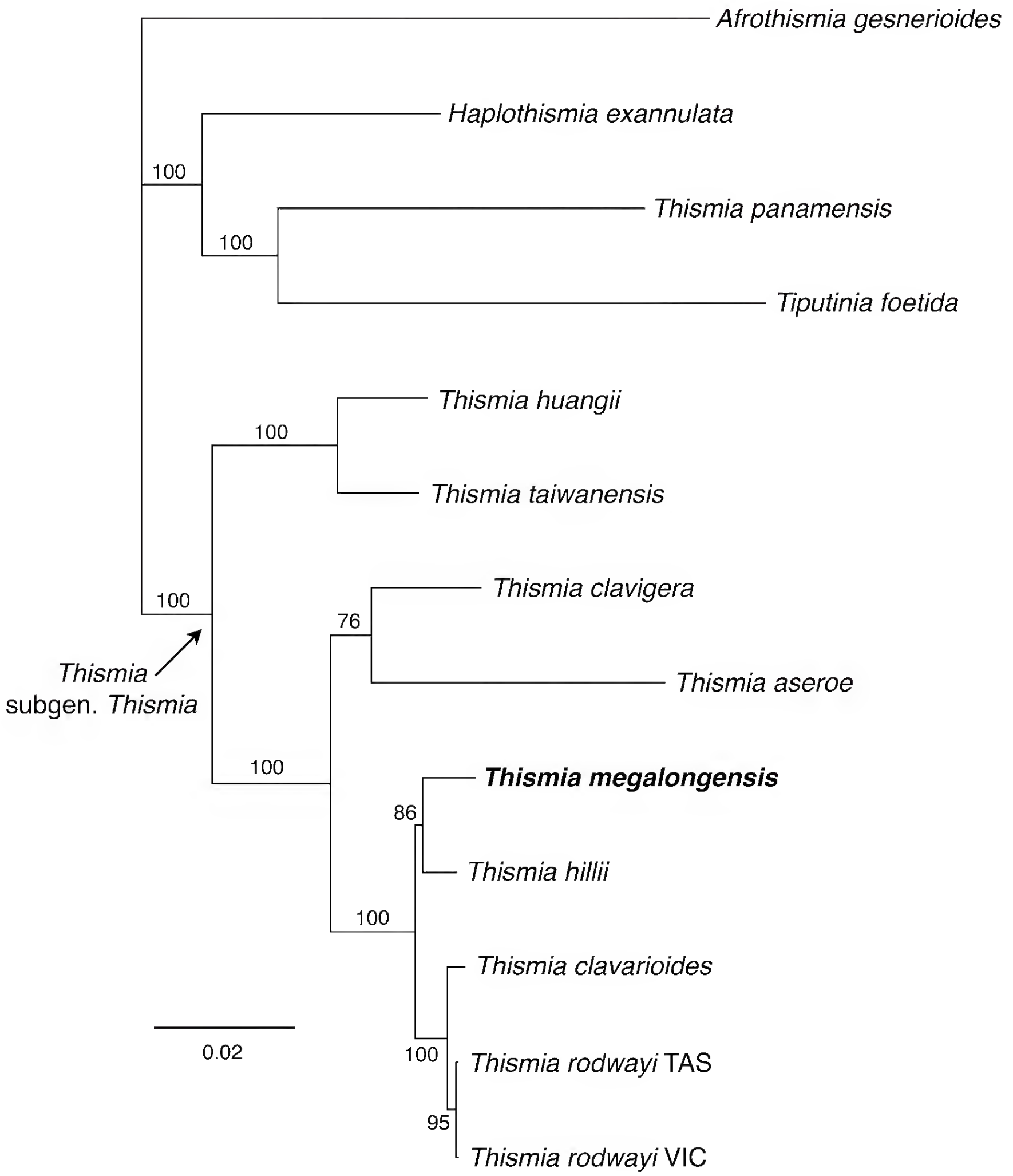

Fig. 5. Majority rule consensus tree obtained with Bayesian analysis of the combined molecular data set (18S rDNA + *atp1*). Bayesian posterior probabilities are indicated at the nodes. Bar represents number of substitutions per site.

Conservation status

A conservation status of 2Kit and distribution of Ni54 Mermaids Cave Recreational Reserve is suggested as per Briggs & Leigh (1996). The IUCN 'Red List' threat code (IUCN 2012) for the species is difficult to determine clearly as the species occurs in only one known site, yet its cryptic nature allows for the potential occurrence elsewhere in this vegetation community. Given the community is widespread, although highly fragmented, the code recommended as a minimum would be VU D1+2, with potentially CR B2ac(iv) applying as a result of the year-on-year observed variation in number of individuals in flower. Additional complexity arises from the unknown extent to which the population is clonal and the longevity of the plants is unknown. It is possible that the species will be severely affected by changes to precipitation, temperature and seasonality of rainfall that are expected to accompany climate change.

Fig. 6. a, Habitat of *Thismia megalongensis*. **b,** and **c,** flowers (arrowed) *in situ* amongst litter and *Blechnum wattsii*.

Key to the currently named Australian species of *Thismia*

1 Mitre without processes; roots coralloid ..... ***T. yorkensis***

1a Mitre with three apical or subapical processes; roots vermiform 2

2 Mitre-processes terminal, spreading or appressed to mitre , < 5 mm long; outer perianth bristle absent, perianth red to orange ..... ***T. rodwayi***

2a Mitre-processes dorsal, erect or spreading, usually > 3 mm long; outer perianth bristle evident 3

3 Outer perianth bristles fine, 3–6 mm long; apical processes fine, 4–6 mm long; perianth red to orange . ..... ***T. megalongensis***

3a Outer perianth bristles thick, 9–11 mm long; apical processes well-developed, 20–25 mm long; perianth very pale or white ..... ***T. clavarioides***

Acknowledgments

The authors thank National Parks and Wildlife Service NSW for permission to collect plant material at Morton National Park and the Megalong Valley. Neville Walsh (MEL) kindly collected DNA material of *Thismia rodwayi* in Victoria, and Mark Wapstra helped collecting DNA of *Thismia* in Tasmania. Financial support was provided to VM by the Netherlands Organisation for Scientific Research (NWO) through a Veni Fellowship (863.11.018).

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Manuscript received 1 July 2014, accepted 28 October 2014

Appendix

Appendix 1.

Genbank accession numbers for the sequences used in the phylogenetic analysis (18S rDNA, apt1).

Thismia hillii (New Zealand): AF309403, AY299849; Thismia megalongensis (Megalong Valley, NSW, Australia – Hunt & Steenbeeke 889871, NSW): KJ885661, KJ885662; Thismia rodwayi (Australia, TAS): KF692536, KF692540; Thismia rodwayi (Australia, VIC): KF692538, KF692541; Thismia huangii (Taiwan): KF692534, KF692543; Thismia taiwanensis (Taiwan): DQ786080, EU421051 ; Thismia clavarioides (Australia): KF692533, KF692539; Thismia clavigera (Malaysia): AF309405, EU421049; Thismia aseroe (Malaysia): AF309404, EU421048; Thismia panamensis (Panama): DQ786081, EU421050; Tiputinia foetida (Ecuador): FJ215764, FJ215770; Haplothismia exannulata (India): DQ786082, EU421037; Afrothismia gesnerioides (Cameroon): EU420989, EU421003.